

**Targeted genotype analyses of GWAS-derived lean body mass and handgrip strength-associated single nucleotide polymorphisms in elite masters athletes**

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**Running head:** Elite master athlete genotype

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27   **Abstract**

28   Recent large genome-wide association studies (GWAS) have independently identified a set of  
29   genetic loci associated with lean body mass (LBM) and handgrip strength (HGS). Evaluation  
30   of these candidate single nucleotide polymorphisms (SNPs) may be useful to investigate  
31   genetic traits of populations at higher or lower risk of muscle dysfunction. As such, we  
32   investigated associations between six SNPs linked to LBM or HGS, in a population of elite  
33   master athletes (MA), and age-matched controls, as a representative population of older  
34   individuals with variable maintenance of muscle mass and function. Genomic DNA was  
35   isolated from buffy coat samples of 96 individuals (consisting of 48 MA (71±6yrs; age-  
36   graded performance 83±9%) and 48 older controls (75±6yrs)). SNP validation and sample  
37   genotyping was conducted using the tetra-primer amplification refractory mutation system  
38   (ARMS). For the 3 SNPs analysed that were previously associated with LBM (*FTO*, *IRS1*  
39   and *ADAMTSL3*), multinomial logistic regression revealed a significant association of the  
40   *ADAMTSL3* genotype with %LBM ( $P<0.01$ ). For the three HGS-linked SNPs, neither *GBF1*  
41   nor *GLIS1* showed any association with HGS, but for *TGFA*, multinomial logistic regression  
42   revealed a significant association of genotype with HGS ( $P<0.05$ ). For *ADAMTSL3*, there  
43   was an enrichment of the effect allele in the MA ( $P<0.05$ ; Fisher's exact test). Collectively,  
44   of the six SNPs analysed, *ADAMTSL3* and *TGFA* showed significant associations with LBM  
45   and HGS, respectively. The functional relevance of the *ADAMTSL3* SNP in body  
46   composition, and of *TGFA* in strength, may highlight a genetic component of the elite MA  
47   phenotype.

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49   **Key words:** muscle; handgrip strength; lean mass; elite athletes

## 50    **Introduction**

51

52    Lean body mass (LBM) plays an important role in metabolic function, mobility and healthy  
53    ageing, where progressive declines in LBM and concurrent increases in lipid infiltration can  
54    have detrimental impacts related to functional impairments and disability (13, 14, 18, 37).  
55    Similarly, declines in muscle strength with ageing are associated with impaired quality-of-life  
56    in older adults and increased risk of frailty and hospitalizations (2, 34). Reflecting this,  
57    handgrip strength (HGS) is a widely used marker of frailty, and a strong predictor of  
58    morbidities and survival (21, 38). The heritability of muscle strength has been estimated to be  
59    between 30-65% (22, 35), with the heritability of the LBM phenotype estimated to be 52-  
60    60% (1, 12). To date, few studies have robustly identified candidate genes associated with  
61    LBM or HGS on a genome-wide level.

62

63    A recent study identified and replicated a set of five loci for total lean body mass (42). Three  
64    of these SNPs (near/in genes for *IRS1*, *ADAMTSL3* and *VCAN*) were also successfully  
65    replicated for appendicular lean mass. Further analyses reported that for a subset of these  
66    SNPs, LBM increasing alleles were associated with adverse metabolic profiles (such as the  
67    Alpha-Ketoglutarate Dependent Dioxygenase (*FTO*) SNP rs9936385), whereas some were  
68    associated with metabolic protection (e.g. the rs2287926 SNP associated with the versican  
69    (*VCAN*) gene) (17). Similarly, a number of recent GWAS have reported multiple loci  
70    associated with HGS (23, 39). Analyses by Matteini *et al.* (2016) identified one significant  
71    genome-wide association of an intergenic SNP located in a chromosomal region that  
72    regulates muscle repair and differentiation. In a study by Willems *et al.* (2017), a number of  
73    loci out of the 16 SNPs identified were related to genes involved in muscle  
74    structure/function; (*ACTG1*), neurotrophic regulation (*TGFA*) and excitation-contraction

75 coupling (*SLC8A1*). Others were identified with less understood roles in muscle function,  
76 such as Golgi Brefeldin A Resistant Guanine Nucleotide Exchange Factor 1 (GBF1), a  
77 guanine nucleotide exchange factor, and GLIS Family Zinc Finger 1 (GLIS1), Kruppel-like  
78 zinc finger protein that regulates transcription. Thus, further investigation into understanding  
79 the roles of these genes in the context of genetic variability of muscle strength is required.  
80 Despite the growing number of GWAS linking candidate genetic loci to skeletal muscle-  
81 related traits in humans, further validation/replication of these SNPs in independent cohorts  
82 has not previously been evaluated, while issues surrounding their reproducibility have also  
83 been highlighted (11).

84

85 Heritable phenotypical traits such as strength and lean mass are undoubtedly associated with  
86 physical performance and thus contribute to elite athletic status (6). Specifically, elite master  
87 athletes (MA; >65yrs) represent a population in which the effects of age may be addressed  
88 independently of the often accompanying disuse (19), and in many cases have displayed  
89 greater neuromuscular function than their age-matched inactive counterparts (24, 27, 29).  
90 However, there are little data available relating genotype to phenotype in these unique  
91 cohorts. In the current study, we first aimed to determine whether associations of SNPs  
92 linked to either LBM or HGS in previous GWAS analyses could be replicated in a smaller  
93 cohort comprising of a mixed population of elite master athletes (MA; both sprint and  
94 endurance) and age-matched non-athlete controls. Secondly, we aimed to compare  
95 allele/genotype frequencies between these two populations in order to gain further insight  
96 into the aforementioned differences in muscular strength and mass between older elite  
97 athletes and their age-matched controls. We hypothesized that the population of MA would  
98 demonstrate greater enrichments in SNPs associated with higher LBM and/or HGS. To  
99 perform targeted genotyping, we used tetra-primer amplification refractory mutation system

100 (ARMS) PCR, which has been reported as a rapid, low-cost and reliable method for SNP  
101 genotyping (26, 40).

## Materials and Methods

### *Participants and ethical approval*

The study was conducted in accordance with the *Declaration of Helsinki*, except for registration in a database. The study was approved by the University Research Ethics Committee and the National Research Ethics Service Committee Northwest (14/NW0275) and (15/NW/0426). All participants provided written informed consent. The control group (n=48) were aged  $75.3 \pm 6.0$  yrs and were recruited from the local community. The masters athletes (n=48) were aged  $70.6 \pm 5.9$  yrs and were recruited from athletics clubs, from an advertisement placed in a national athletics magazine, and from two national masters athletics competitions as part of the wider Vertical Impact of Bone Health in Elderly (VIBE) multiple cohort study (5, 28). All masters athletes were actively competing in their respective disciplines, and all completed more than 5 hours of specific training per week at the time of testing. MAs were classified as sprinters (n=12) if competing in events less than 800 m in distance, or endurance athletes (n=36) if competing in events greater than or equal to 800 m in distance.

The age-graded performance (AGP) of a master athlete allows a comparison of current performance against world record performance in the same discipline, distance and age-group. Mean age-graded performance (AGP) was determined by taking the athlete's highest ranked performance within the last year and expressing it as a percentage of the world record for that age and distance. The mean AGP of this athletic cohort was  $83.4 \pm 8.6\%$ . For example, a 21 min and 20 sec 5000m for a 70-year-old man gives an age-graded performance of 83%. All males were chosen for the current analysis in order to avoid influences of sex-specific hormones.

127

128 **DXA Scans**

129 Standing height was measured to the nearest millimeter and body mass was measured to the  
130 nearest 0.1 kg. Whole body, total hip and lumbar spine dual energy X-ray absorptiometry  
131 (DXA: Lunar Prodigy Advanced, GE Healthcare, encore version 10.50.086, London, UK)  
132 scans were performed while the participant lay supine wearing a light cotton t-shirt to reduce  
133 measurement errors due to clothing absorption. Lean mass was taken from results of total  
134 body scans and regional analysis of legs and arms. All measurements were recorded after  
135 manual adjustment of the regions of interest. Repeat total body scans were performed in 8  
136 participants within one month of the first scan. Using these repeat scans, the short-term error  
137 for our laboratory was 0.01% for whole body lean mass.

138

139 **Muscle function**

140 The investigators provided verbal instructions and a physical demonstration of the muscle  
141 function tests. Participants were allowed one practice immediately before the actual assessed  
142 trials, which acted as a specific warm up and also confirmed that the instructions were  
143 understood. In all cases, the muscle function tests were completed between 10am and 3pm.

144

145 Hand grip strength was measured using the Jamar dynamometer handle (Sammons Preston  
146 Inc, Bolingbrook, IL, USA) as previously described (10). The width of the dynamometer was  
147 adjusted for each participant separately. Participants were instructed to stand upright with the  
148 arm fully extended along the body, maintaining approximately 5 cm gap between the wrist  
149 and the hip or upper leg (so that the hand was not rested against the body). Participants were  
150 instructed to squeeze against the handle as hard possible for three seconds. Grip strength was

measured three times and recorded in kilograms to the nearest 0.1 kg. For the purpose of this study, the best of three attempts was included in further analysis.

A Leonardo Jump Mechanography Platform (Leonardo Software version 4.2: Novotiec Medical GmbH, Pforzheim, Germany) was used to assess lower limb muscle power during a countermovement vertical jump, as described previously (10). Results for both absolute (W) and relative (W/kg) power were recorded. Briefly, a two-footed countermovement jump was performed starting with feet approximately 30 cm apart (slightly narrower than shoulder width) and standing upright on the force plates. Force was sampled at 800 Hz. Participants flexed at the knees before extending as forcefully as possible to take off for the jump. Jumps were performed with a trained research assistant in close proximity to intervene in case of a trip or fall. Each participant repeated the jump sequence three times, with approximately 60 seconds rest between efforts. The jump with the highest value for power was used for statistical analysis.

#### *Genomic DNA Extraction*

Genomic DNA was extracted from buffy coat samples (200 µl) using the QIAamp blood mini DNA kit (Qiagen, UK), according to the manufacturer's instructions. Isolated DNA was quantified on the NanoDrop 2000 (Thermo Fisher Scientific, UK).

#### *SNP selection and primer design*

A set of SNPs were selected, chosen from SNPs previously linked with LBM (42) and HGS in humans (39). SNPs with very low/high effect allele frequencies (EAFs) in the original GWAS studies (e.g. *VCAN*, *KANSL1* and *POLD3*) were avoided due to expected difficulties in detecting them in relatively low sample sizes. Primer design was performed using the



PRIMER1 program: <http://primer1.soton.ac.uk/primer1.html>, using the default primer design settings. SNPs that yielded primers with very high GC content were avoided due to anticipated difficulties during amplification, as well as primer sets with very distinct melting temperatures. A total of 15 SNPs were initially tested for validation, however technical difficulties meant that a number could not be assessed with the tetra-primer ARMS PCR method, and the final set of six SNPs, three predicted to be associated with LBM and three with HGS, are presented in Table 1.

#### *Tetra-primer ARMS PCR and gel electrophoresis*

Validation of SNP primers and genotyping was performed using the tetra-primer ARMS PCR technique (40). The sequences of primers used for the genotyping of the selected SNPs are shown in Table 1. SNP primers were initially validated and optimised using the guidelines set out in (26). Initially, amplification was performed using the outer primers only, using a gradient annealing temperature PCR to determine the optimal annealing temperature for each primer set. Subsequent validation involved incorporating the inner primers in varying amounts to produce detectable bands for each allele-specific amplicon via agarose gel electrophoresis (see below). PCR reactions with a final volume of 18 µl including 30 ng genomic DNA, SYBR™ Select Master Mix (Applied Biosystems) and primers in ratios according to Table 1. Amplification was performed using a Viia™ 7 real-time PCR machine (Applied Biosystems), using the following cycling conditions: 1 cycle of initial denaturation at 95°C, 2 min; 35 cycles of denaturation at 95°C for 30s, annealing at 61-62°C (see Table 1 for SNP-specific annealing temperature) for 45s and extension at 72°C for 45s, with a final extension for 5 min at 72°C on standard cycling conditions. PCR products were mixed with 4 µl gel loading buffer (Sigma-Aldrich, UK) and 10 µl was electrophoresed on 3% (w/v) agarose gels for 120 min at 80V.

201

202 *Statistical analyses*

203 Multinomial logistic regression was performed in R (version 3.6.1) using the nnet package  
204 (16) to examine associations between *GBF1*, *GLIS1* and *TGFA* genotypes and maximal grip  
205 strength, and to examine associations between *IRS1*, *FTO* and *ADAMTSL3* and total lean  
206 mass, appendicular lean mass, and percentage body lean mass. Strength of associations were  
207 assessed by p values calculated from z values provided from the regression model  
208 coefficients and standard errors for each predictor variable. Fisher's exact test was used for  
209 comparison of allele distributions and genotype distributions between MA and control  
210 groups, while one-way ANOVA was used for multi-group comparisons, with Tukey's test to  
211 correct for multiple comparisons. Comparisons between two groups were made using  
212 unpaired t tests.  $P < 0.05$  was taken to be statistically significant. Data were analysed using  
213 GraphPad Prism software version 7.0.

## Results

### *Associations between genotype and functional parameters in MA and non-athletes*

We first aimed to identify any associations of the selected SNP genotypes with lean mass or HGS in a mixed population of older MA and non-athletes (i.e., irrespective of groupings). Within this cohort, total LBM ranged from 36.6 to 69.4 kg, while HGS ranged from 20.6 to 54.7 kg. In relation to the SNPs previously linked to HGS (*GBF1* (rs2273555; effect allele A), *GLIS1* (rs4926611; effect allele C) and Transforming Growth Factor Alpha (*TGFA*; rs958685; effect allele A)), there was no significant association of either *GBF1* or *GLIS1* genotype with HGS (Figure 1), but with *TGFA*, there was a significant association between HGS and genotype (mean difference between AA and CC 6.32; 95% CI 0.43-12.1;  $P<0.05$ , Figure 1A), with the AA genotype (A being the effect allele) having higher HGS. In relation to SNPs that were previously associated with LBM, multinomial logistic regression showed no significant association of total lean mass, % LBM or appendicular lean mass with insulin receptor substrate 1 (*IRS1*; rs2943656; effect allele A), *FTO* (rs9936385; effect allele T) or A Disintegrin-Like And Metalloprotease Domain With Thrombospondin Type I Motifs-Like 3 (*ADAMTSL3*; rs4842924; effect allele T) genotypes (Figures 2-4). For *ADAMTSL3* however, there was a significant association with % LBM (mean difference between TT and CC 5.36; 95% CI 1.38-9.34;  $P<0.01$ ; Figure 4A), where the TT genotype was associated with higher % LBM. Since LBM and HGS are biologically closely related, we also determined whether any of the LBM-associated SNPs were linked to HGS, and vice-versa. However, none of the HGS-associated SNPs were significantly associated with LBM (*TGFA*;  $\beta=-4.88$ ,  $p=0.305$  *GLIS1*;  $\beta=-18.64$ ,  $p=0.641$ , *GBF1*;  $\beta=2.433$ ,  $p=0.354$ ), and none of the LBM-associated SNPs were associated with HGS (*FTO*;  $\beta=-1.716$ ,  $p=0.354$ , *IRS1*;  $\beta=-3.242$ ,  $p=0.059$ , *ADAMTSL3*;  $\beta=-1.432$ ,  $p=0.378$ ). There were also no genotype associations of any of the SNPs measured

with muscle power measurements (maximum power relative to body weight,  $P_{max\ rel}$ ) (*TGFA*;  $\beta=-0.079$ ,  $p=0.714$ , *GLIS1*;  $\beta=-0.002$ ,  $p=0.911$ , *GBF1*;  $\beta=-0.006$ ,  $p=0.891$ , *FTO*;  $\beta=-0.021$ ,  $p=0.358$ , *IRS1*;  $\beta=-0.002$ ,  $p=0.905$ , *ADAMTSL3*;  $\beta=0.015$ ,  $p=0.423$ ).

#### *Allele frequencies in individuals grouped according to the highest and lowest quartile for % LBM or HGS.*

Following on from this, we aimed to determine whether there were any differences in allele frequencies in individuals that had been grouped according to the highest and lowest quartiles for % LBM or HGS. Comparing the upper and lower quartiles for %LBM (irrespective of groupings) there was no difference in allele frequency for the *IRS1* or *FTO* SNPs (Table 2). For *ADAMTSL3*, comparing the upper and lower quartiles for %LBM (irrespective of groupings), there was an enrichment in the effect allele in the upper quartile for %LBM ( $P<0.05$ ; Fisher's exact test) (Table 2). For *TGFA*, comparing the upper and lower quartiles for HGS (irrespective of groupings), there was an enrichment in the effect allele in the upper quartile for HGS ( $P<0.05$ ; Fisher's exact test) (Table 2). There were no significant differences in either *GBF1* or *GLIS1* alleles between the upper and lower quartiles for HGS (Table 2).

#### *Allele/genotype distributions for LBM or HGS-associated SNPs in MA versus non-athletes*

In subsequent analyses, we sought to compare allele/genotype distributions for the LBM and HGS-associated SNPs between the elite MA and older non-athlete groups, first comparing participant muscle-related characteristics between MA and control groups. Since multiple group analyses were limited by the relatively low number of available samples from participants in the sprint category ( $n=12$ ), sprint and endurance MA were grouped for the majority of our analyses. While total lean mass and appendicular lean mass (ALM) was not

different across groups (Figure 5A & B), LBM as a percentage of total body weight (%LBM) was significantly lower in controls than MA ( $P<0.001$  by unpaired t test; Figure 5C). Likewise, percentage fat mass was significantly higher ( $P<0.001$  by unpaired t test) in controls than MA (Figure 5D). HGS and Pmax rel were no different between MA and controls (Figure 5E and 5F).

Genotype distributions for 3 SNPs that were previously associated with LBM (*IRS1*, *FTO* and *ADAMTSL3*) and 3 SNPs that were previously associated with HGS (*TGFA*, *GBF1* and *GLIS1*) were analysed in the 48 MA and 48 older controls. For the SNP associated with the *ADAMTSL3* gene, genotype distributions were significantly different between MA and controls ( $P<0.05$ ; Fisher's exact test; Figure 6). For the SNPs associated with *IRS1*, *FTO*, *TGFA*, *GLIS1* and *GBF1*, there was no difference in genotype frequencies between MA and control groups (Figure 6). While analyses focused on the master athletes as a group, compared to non-athlete control, we also assessed allele distributions for the 6 SNPs between sprint and endurance MA relative to controls (Table 3). Similar to the genotype distributions between MA and controls, allele distributions for the SNPs associated with *IRS1*, *FTO*, *TGFA*, *GLIS1* and *GBF1* were not significantly different between groups, while for *ADAMTSL3*, there was an enrichment in the effect allele for both sprint and endurance athletes, relative to non-athlete controls ( $P<0.05$  vs. Control (Fisher's exact test); Table 3).

## Discussion

While LBM and HGS represent two highly heritable traits in humans (1, 22, 35), only recently have studies begun to explore the specific genes that contribute to the underlying inter-individual variability in skeletal muscle traits such as these (30, 39, 42). Evaluation of these candidate SNPs could prove useful in investigating underlying genetic traits of individuals at variable risk of muscle dysfunction. In the present study, our aim was to determine whether SNPs linked to either LBM or HGS in previous GWAS analyses could be replicated in a smaller cohort comprising of elite MA and age-matched controls. We also aimed to determine whether genotype/allele distributions for these SNPs were different between elite MA in comparison to age-matched non-exercising controls, as a representative population of older individuals with greater maintenance of muscle mass and function. By comparing allele/genotype frequencies between these two populations using the tetra-primer ARMS technique we aimed to gain greater insights into the underlying genetic component of the MA muscle phenotype.

We chose to use the tetra-primer ARMS technique as a rapid approach to SNP genotyping as it provides a cost-effective and accurate methodology, (40) but alternative methods are available. The restriction fragment length polymorphism (RFLP) typing method involves restriction endonuclease digestion of PCR products to discriminate between alleles (25), while microarray approaches (32) and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (8) allow high-throughput genotyping. We found the tetra-primer ARMS technique robust, but requiring substantial optimisation, and some primer sets for SNPs could not be validated; potentially due to the SNPs loci i.e. in a high

GC-rich region, giving rise to difficulties due to incomplete denaturation of DNA and less than optimal primer annealing (26).

We began by investigating associations between genotype and functional parameters in older MA and non-athletes as a collective cohort. In terms of their predicted associations with either LBM or HGS, out of the six SNPs analysed, four failed to show any significant association with LBM or HGS (even when analysing those individuals with the highest and lowest quartiles for %LBM or HGS). In contrast, the SNP associated with *TGFA* showed significant associations with HGS, while the SNP linked with *ADAMTSL3* was associated with LBM (independent of exercise discipline), as predicted by the original GWAS'. These findings provide further support to the previous data indicating the potential importance of the *TGFA* SNP in muscle strength, and of *ADAMTSL3* in body composition. Interestingly, we found that none of the HGS-associated SNPs were associated with LBM, and vice-versa, nor were there any significant associations with Pmax rel. The reason for this lack of overlap is not clear and requires further investigation of the potential roles of these genes in muscle function. For the SNP associated with *TGFA*, there was an association between HGS and genotype, with the AA genotype (A being the effect allele promoting increased HGS), having a significantly higher HGS. The consequence of the polymorphism with rs958685 is an intron variant. The potential functional relevance of the *TGFA* in muscular strength remains to be evaluated, but other intronic SNPs have been shown to be associated with functional elements, including intron splicing enhancers/silencers that regulate alternative splicing events as well as other transcriptional regulatory elements (4). The *TGFA* gene encodes a growth factor which plays a key role in cellular proliferation, differentiation and development (33). TGF- $\alpha$  also plays a neurotrophic role and promotes neuronal survival during acute injury of motor neurons (15, 20).

333

334 A further important finding was that for rs4842924, the SNP related to the *ADAMTSL3* gene,  
335 the TT genotype was associated with higher %LBM amongst all volunteers. Initial analyses  
336 aimed to replicate the original GWAS (42), which identified SNPs associated with total  
337 LBM, with subsequent analysis demonstrating higher associations when adjusting for total fat  
338 mass (17). We found instead that for *ADAMTSL3* (and other SNPs), there was no association  
339 to LBM in either unadjusted or after adjusting for fat mass or for height. We also found no  
340 associations of any of the SNPs to appendicular lean mass. There was, however, a significant  
341 association of the *ADAMTSL3* genotype to LBM as a percentage of whole-body mass,  
342 demonstrating it may have importance in terms of body composition. As with *TGFA*, the  
343 consequence of the *ADAMTSL3* SNP is an intron variant, and the functional effect (if any) on  
344 gene expression is not currently known. Little is understood about the biological functions of  
345 *ADAMTSL3*, but it is a glycoprotein that is related to the ADAMTS family of  
346 metalloproteases, that may have functions in extracellular matrix regulation (9). The  
347 *ADAMTSL3* gene has also consistently been linked to height (36) in genome-wide association  
348 analyses. Further *in vitro* experiments will be required to understand the mechanisms  
349 underlying *ADAMTSL3* gene variants in muscle physiology, and relation to LBM *in vivo*.

350

351 We next investigated allele/genotype distributions for LBM or HGS-associated SNPs in MA  
352 versus non-athletes. Elite MA represent a unique population of individuals that in general  
353 display greater maintenance of neuromuscular function than age-matched inactive  
354 populations (24), and while undoubtedly environmental factors play a large role in the MA  
355 phenotype (7), there are little conclusive data available related to any underlying genetic  
356 components. Whether the high-functioning characteristics of master athletes is more  
357 influenced by heritable factors regulating muscle composition/performance, or whether the



environmental component (i.e. continued high levels of training over the years) is more important for the master athlete phenotype, remains to be fully understood. For the present group of individuals studied, while total LBM or ALM were not different between MA and controls, %LBM was significantly higher in the MA population. While HGS or Pmax Rel were not different between MA and non-athlete controls, this is likely due to the fact that the majority of the cohort were endurance athletes, which is in line with previous observations with regards to strength differences in endurance versus power MA (24). Although HGS does not always correlate with strength of other functionally important muscle groups such as the quadriceps (41), it is a useful predictor of a number of health outcomes in middle to older age (3), including all cause mortality (31). In the present study, of the six SNPs measured, five were not different between MA and control; however, for *ADAMTSL3*, there was an enrichment of the effect allele (T) in the group of MA. Further work investigating these candidate SNPs, and the mechanisms by which they may influence muscle function, could prove useful in understanding the genetic basis of populations with increased/decreased susceptibility of muscle dysfunction (such as frailty and sarcopenia).

### *Perspectives and Significance*

While there are difficulties associated with studying a cohort such as that of the MA in terms of gaining sufficient sample numbers, clearly larger MA sample sizes will be needed to explore MA, on a genome-wide basis, or in a targeted fashion. Indeed, the lack of individuals with the GG genotype for *IRS1* in the present study is also a limitation in the context of the relatively small sample size of this study. There is also a potential that the lack of replication for some of the SNPs analysed in the present study was partly due to the the elite athletes having a different phenotype to those of the general population (as used in the original GWAS analyses). Additionally, effect sizes in the original analyses would be viewed as being

small, with standardized beta of -0.12 - -0.14 for LBM and 0.13 – 0.16 for HGS. More work is required to determine the biological significance of these SNPs in LBM and/or muscular strength across different populations of individuals. Nonetheless, in a targeted fashion, we demonstrate that a SNP related to the *ADAMTSL3* gene was enriched in elite MA and had significant associations with % LBM. We also confirmed data from previous GWAS' of an association of the *TGFA* SNP with HGS. Future work elucidating the mechanisms by which these gene variants influence muscle mass and function are required to facilitate our understanding of the genetic basis of, not only the MA phenotype, but also the genetic basis underlying a range of conditions such as frailty and sarcopenia.

**Acknowledgements**

The authors would like to thank the participants for their involvement in this study. This work was supported by funding from the UK Medical Research Council as part of the Life Long health and Wellbeing initiative (MR/K025252/1). This work was supported by the Medical Research Council [grant number MR/P021220/1] as part of the MRC-ARUK Centre for Musculoskeletal Ageing Research awarded to the Universities of Nottingham and Birmingham, and supported by the NIHR Nottingham Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

**Disclosures**

The authors declare they have no competing interests.

## References

1. **Arden NK, Spector TD.** Genetic influences on muscle strength, lean body mass, and bone mineral density: A twin study. *J Bone Miner Res* 12: 2076–2081, 1997.
2. **Bohannon RW.** Hand-grip dynamometry predicts future outcomes in aging adults. *J Geriatr Phys Ther* 31: 3–10, 2008.
3. **Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, Iliodromiti S, Sillars A, Graham N, MacKay DF, Pell JP, Gill JMR, Sattar N, Gray SR.** Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: Prospective cohort study of half a million UK Biobank participants. *BMJ* (2018). doi: 10.1136/bmj.k1651.
4. **Cooper DN.** Functional intronic polymorphisms: Buried treasure awaiting discovery within our genes. *Hum Genomics* 4: 284–288, 2010.
5. **Deere KC, Hannam K, Coulson J, Ireland A, McPhee JS, Moss C, Edwards MH, Dennison E, Cooper C, Sayers A, Lipperts M, Grimm B, Tobias JH.** Quantifying habitual levels of physical activity according to impact in older people: Accelerometry protocol for the VIBE study. *J Aging Phys Act* 24: 290–295, 2016.
6. **Eynon N, Ruiz JR, Oliveira J, Duarte JA, Birk R, Lucia A.** Genes and elite athletes: A roadmap for future research. *J. Physiol.* 589: 3063–3070, 2011.
7. **Georgiades E, Klissouras V, Baulch J, Wang G, Pitsiladis Y.** Why nature prevails over nurture in the making of the elite athlete. *BMC Genomics* 18: 2017.
8. **Griffin TJ, Smith LM.** Single-nucleotide polymorphism analysis by MALDI-TOF mass spectrometry. *Trends Biotechnol.* 18: 77–84, 2000.
9. **Hall NG, Klenotic P, Anand-Apte B, Apte SS.** ADAMTSL-3/punctin-2, a novel glycoprotein in extracellular matrix related to the ADAMTS family of metalloproteases. *Matrix Biol* 22: 501–510, 2003.

- 432 10. **Hannam K, Hartley A, Clark EM, Sayer AA, Tobias JH, Gregson CL.** Feasibility  
433 and acceptability of using jumping mechanography to detect early components of  
434 sarcopenia in community-dwelling older women. *J Musculoskelet Neuronal Interact*  
435 17: 246–257, 2017.
- 436 11. **Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K.** A comprehensive review  
437 of genetic association studies. *Genet. Med.* 4: 45–61, 2002.
- 438 12. **Hsu FC, Lenchik L, Nicklas BJ, Lohman K, Register TC, Mychaleckyj J,**  
439 **Langefeld CD, Freedman BI, Bowden DW, Carr JJ.** Heritability of body  
440 composition measured by DXA in the Diabetes Heart Study. *Obes Res* 13: 312–319,  
441 2005.
- 442 13. **Janssen I.** Influence of sarcopenia on the development of physical disability: The  
443 cardiovascular health study. *J Am Geriatr Soc* 54: 56–62, 2006.
- 444 14. **Janssen I, Heymsfield SB, Ross R.** Low relative skeletal muscle mass (sarcopenia) in  
445 older persons is associated with functional impairment and physical disability. *J Am*  
446 *Geriatr Soc* 50: 889–896, 2002.
- 447 15. **Junier MP.** What role(s) for TGF $\alpha$  in the central nervous system? *Prog. Neurobiol.*  
448 62: 443–473, 2000.
- 449 16. **Kafadar K, Koehler JR, Venables WN, Ripley BD.** Modern Applied Statistics with  
450 S-Plus. 1999.
- 451 17. **Karasik D, Zillikens MC, Hsu YH, Aghdassi A, Akesson K, Amin N, Barroso I,**  
452 **Bennett DA, Bertram L, Bochud M, Borecki IB, Broer L, Buchman AS, Byberg**  
453 **L, Campbell H, Campos-Obando N, Cauley JA, Cawthon PM, Chambers JC,**  
454 **Chen Z, Cho NH, Choi HJ, Chou WC, Cummings SR, De Groot LCPGM, De**  
455 **Jager PL, Demuth I, Diatchenko L, Econs MJ, Eiriksdottir G, Enneman AW,**  
456 **Eriksson J, Eriksson JG, Estrada K, Evans DS, Feitosa MF, Fu M, Gieger C,**

457 **Grallert H, Gudnason V, Lenore LJ, Hayward C, Hofman A, Homuth G,**  
 458 **Huffman KM, Husted LB, Illig T, Ingelsson E, Ittermann T, Jansson JO, Johnson**  
 459 **T, Biffar R, Jordan JM, Jula A, Karlsson M, Khaw KT, Kilpeläinen TO, Klopp**  
 460 **N, Kloth JSL, Koller DL, Kooner JS, Kraus WE, Kritchevsky S, Kutalik Z,**  
 461 **Kuulasmaa T, Kuusisto J, Laakso M, Lahti J, Lang T, Langdahl BL, Lerch MM,**  
 462 **Lewis JR, Lill C, Lind L, Lindgren C, Liu Y, Livshits G, Ljunggren Ö, Loos RJF,**  
 463 **Lorentzon M, Luan J, Luben RN, Malkin I, McGuigan FE, Medina-Gomez C,**  
 464 **Meitinger T, Melhus H, Mellström D, Michaëlsson K, Mitchell BD, Morris AP,**  
 465 **Mosekilde L, Nethander M, Newman AB, Oconnell JR, Oostra BA, Orwoll ES,**  
 466 **Palotie A, Peacock M, Perola M, Peters A, Prince RL, Psaty BM, Räikkönen K,**  
 467 **Ralston SH, Ripatti S, Rivadeneira F, Robbins JA, Rotter JI, Rudan I, Salomaa**  
 468 **V, Satterfield S, Schipf S, Shin CS, Smith A V., Smith SB, Soranzo N, Spector**  
 469 **TD, StanĀ Āková A, Stefansson K, Steinhagen-Thiessen E, Stolk L, Streeten EA,**  
 470 **Styrkarsdottir U, Swart KMA, Thompson P, Thomson CA, Thorleifsson G,**  
 471 **Thorsteinsdottir U, Tikkanen E, Tranah GJ, Uitterlinden AG, Van Duijn CM,**  
 472 **Van Schoor NM, Vandenput L, Vollenweider P, Völzke H, Wactawski-Wende J,**  
 473 **Walker M, J Wareham N, Waterworth D, Weedon MN, Wichmann HE, Widen**  
 474 **E, Williams FMK, Wilson JF, Wright NC, Yerges-Armstrong LM, Yu L, Zhang**  
 475 **W, Zhao JH, Zhou Y, Nielson CM, Harris TB, Demissie S, Kiel DP, Ohlsson C.**  
 476 Disentangling the genetics of lean mass. *Am J Clin Nutr* 109: 276–278, 2019.  
 477 18. **Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, Corsi**  
 478 **AM, Rantanen T, Guralnik JM, Ferrucci L.** Age-associated changes in skeletal  
 479 muscles and their effect on mobility: An operational diagnosis of sarcopenia. *J Appl*  
 480 *Physiol* 95: 1851–1860, 2003.  
 481 19. **Lazarus NR, Lord JM, Harridge SDR.** The relationships and interactions between

- age, exercise and physiological function. *J. Physiol.* 597: 1299–1309, 2019.
20. **Lisovsky F, Blot S, Lacombe C, Bellier JP, Dreyfus PA, Junier MP.** Transforming growth factor  $\alpha$  expression as a response of murine motor neurons to axonal injury and mutation-induced degeneration. *J Neuropathol Exp Neurol* 56: 459–471, 1997.
21. **Marsh AP, Rejeski WJ, Espeland MA, Miller ME, Church TS, Fielding RA, Gill TM, Guralnik JM, Newman AB, Pahor M.** Muscle strength and BMI as predictors of major mobility disability in the lifestyle interventions and independence for elders pilot (LIFE-P). *Journals Gerontol - Ser A Biol Sci Med Sci* 66 A: 1376–1383, 2011.
22. **Matteini AM, Fallin MD, Kammerer CM, Schupf N, Yashin AI, Christensen K, Arbeev KG, Barr G, Mayeux R, Newman AB, Walston JD.** Heritability estimates of endophenotypes of long and health life: The long life family study. *Journals Gerontol - Ser A Biol Sci Med Sci* 65 A: 1375–1379, 2010.
23. **Matteini AM, Tanaka T, Karasik D, Atzmon G, Chou WC, Eicher JD, Johnson AD, Arnold AM, Callisaya ML, Davies G, Evans DS, Holtfreter B, Lohman K, Lunetta KL, Mangino M, Smith A V., Smith JA, Teumer A, Yu L, Arking DE, Buchman AS, Chibinik LB, DeJager PL, Evans DA, Faul JD, Garcia ME, Gillham-Nasenya I, Gudnason V, Hofman A, Hsu YH, Ittermann T, Lahousse L, Liewald DC, Liu Y, Lopez L, Rivadeneira F, Rotter JI, Siggeirsdottir K, Starr JM, Thomson R, Tranah GJ, Uitterlinden AG, Völker U, Völzke H, Weir DR, Yaffe K, Zhao W, Zhuang WV, Zmuda JM, Bennett DA, Cummings SR, Deary IJ, Ferrucci L, Harris TB, Kardina SLR, Kocher T, Kritchevsky SB, Psaty BM, Seshadri S, Spector TD, Srikanth VK, Windham BG, Zillikens MC, Newman AB, Walston JD, Kiel DP, Murabito JM.** GWAS analysis of handgrip and lower body strength in older adults in the CHARGE consortium. *Aging Cell* 15: 792–800, 2016.
24. **Mckendry J, Breen L, Shad BJ, Greig CA.** Muscle morphology and performance in

507 master athletes: A systematic review and meta-analyses. *Ageing Res. Rev.* 45: 62–82,  
508 2018.

509 25. **Medrano JF, Aguilar-Cordova E.** Polymerase Chain Reaction Amplification of  
510 Bovine  $\beta$ -Lactoglobulin Genomic Sequences and Identification of Genetic Variants by  
511 Relp Analysis. *Anim Biotechnol* 1: 73–77, 1990.

512 26. **Medrano RFV, De Oliveira CA.** Guidelines for the tetra-primer ARMS-PCR  
513 technique development. *Mol Biotechnol* 56: 599–608, 2014.

514 27. **Piasecki J, Ireland A, Piasecki M, Deere K, Hannam K, Tobias J, McPhee JS.**  
515 Comparison of muscle function, bone mineral density and body composition of early  
516 starting and later starting older masters athletes. *Front Physiol* 10, 2019.

517 28. **Piasecki J, McPhee JS, Hannam K, Deere KC, Elhakeem A, Piasecki M, Degens**  
518 **H, Tobias JH, Ireland A.** Hip and spine bone mineral density are greater in master  
519 sprinters, but not endurance runners compared with non-athletic controls. *Arch*  
520 *Osteoporos* 13, 2018.

521 29. **Piasecki M, Ireland A, Piasecki J, Degens H, Stashuk DW, Swiecicka A, Rutter**  
522 **MK, Jones DA, McPhee JS.** Long-term endurance and power training may facilitate  
523 motor unit size expansion to compensate for declining motor unit numbers in older  
524 age. *Front Physiol* 10, 2019.

525 30. **Roth SM.** Genetic aspects of skeletal muscle strength and mass with relevance to  
526 sarcopenia. *Bonekey Rep* 1, 2012.

527 31. **Sasaki H, Kasagi F, Yamada M, Fujita S.** Grip Strength Predicts Cause-Specific  
528 Mortality in Middle-Aged and Elderly Persons. *Am. J. Med.* (2007). doi:  
529 10.1016/j.amjmed.2006.04.018.

530 32. **Shen R, Fan JB, Campbell D, Chang W, Chen J, Doucet D, Yeakley J, Bibikova**  
531 **M, Garcia EW, McBride C, Steemers F, Garcia F, Kermani BG, Gunderson K,**



- 532 **Oliphant A.** High-throughput SNP genotyping on universal bead arrays. *Mutat. Res. -*  
533 *Fundam. Mol. Mech. Mutagen.* 573: 70–82, 2005.
- 534 33. **Singh B, Coffey RJ.** From wavy hair to naked proteins: The role of transforming  
535 growth factor alpha in health and disease. *Semin. Cell Dev. Biol.* 28: 12–21, 2014.
- 536 34. **Taekema DG, Gussekloo J, Maier AB, Westendorp RGJ, de Craen AJM.**  
537 Handgrip strength as a predictor of functional, psychological and social health. A  
538 prospective population-based study among the oldest old. *Age Ageing* 39: 331–337,  
539 2010.
- 540 35. **Tiainen K, Sipilä S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A,**  
541 **Pajala S, Rantanen T.** Heritability of maximal isometric muscle strength in older  
542 female twins. *J Appl Physiol* 96: 173–180, 2004.
- 543 36. **Weedon MN, Lango H, Lindgren CM, Wallace C, Evans DM, Mangino M,**  
544 **Freathy RM, Perry JRB, Stevens S, Hall AS, Samani NJ, Shields B, Prokopenko**  
545 **I, Farrall M, Dominiczak A, Johnson T, Bergmann S, Beckmann JS,**  
546 **Vollenweider P, Waterworth DM, Mooser V, Palmer CNA, Morris AD,**  
547 **Ouwehand WH, Zhao JH, Li S, Loos RJJ, Barroso I, Deloukas P, Sandhu MS,**  
548 **Wheeler E, Soranzo N, Inouye M, Wareham NJ, Caulfield M, Munroe PB,**  
549 **Hattersley AT, McCarthy MI, Frayling TM.** Genome-wide association analysis  
550 identifies 20 loci that influence adult height. *Nat Genet* 40: 575–583, 2008.
- 551 37. **Wilkinson DJ, Piasecki M, Atherton PJ.** The age-related loss of skeletal muscle  
552 mass and function: Measurement and physiology of muscle fibre atrophy and muscle  
553 fibre loss in humans. *Ageing Res Rev* 47: 123–132, 2018.
- 554 38. **Willcox BJ, He Q, Chen R, Yano K, Masaki KH, Grove JS, Donlon TA, Willcox**  
555 **DC, Curb JD.** Midlife risk factors and healthy survival in men. *J Am Med Assoc* 296:  
556 2343–2350, 2006.

557 39. Willems SM, Wright DJ, Day FR, Trajanoska K, Joshi PK, Morris JA, Matteini  
 558 AM, Garton FC, Grarup N, Oskolkov N, Thalamuthu A, Mangino M, Liu J,  
 559 Demirkan A, Lek M, Xu L, Wang G, Oldmeadow C, Gaulton KJ, Lotta LA,  
 560 Miyamoto-Mikami E, Rivas MA, White T, Loh PR, Aadahl M, Amin N, Attia JR,  
 561 Austin K, Benyamin B, Brage S, Cheng YC, Ciężczyk P, Derave W, Eriksson  
 562 KF, Eynon N, Linneberg A, Lucia A, Massidda M, Mitchell BD, Miyachi M,  
 563 Murakami H, Padmanabhan S, Pandey A, Papadimitriou I, Rajpal DK, Sale C,  
 564 Schnurr TM, Sessa F, Shrine N, Tobin MD, Varley I, Wain L V., Wray NR,  
 565 Lindgren CM, MacArthur DG, Waterworth DM, McCarthy MI, Pedersen O,  
 566 Khaw KT, Kiel DP, Pitsiladis Y, Fuku N, Franks PW, North KN, Van Duijn CM,  
 567 Mather KA, Hansen T, Hansson O, Spector T, Murabito JM, Richards JB,  
 568 Rivadeneira F, Langenberg C, Perry JRB, Wareham NJ, Scott RA, Oei L, Zheng  
 569 HF, Forgetta V, Leong A, Ahmad OS, Laurin C, Mokry LE, Ross S, Elks CE,  
 570 Bowden J, Warrington NM, Murray A, Ruth KS, Tsilidis KK, Medina-Gómez C,  
 571 Estrada K, Bis JC, Chasman DI, Demissie S, Enneman AW, Hsu YH, Ingvarsson  
 572 T, Kähönen M, Kammerer C, Lacroix AZ, Li G, Liu CT, Liu Y, Lorentzon M,  
 573 Mägi R, Mihailov E, Milani L, Moayyeri A, Nielson CM, Sham PC, Siggeirsdottir  
 574 K, Sigurdsson G, Stefansson K, Trompet S, Thorleifsson G, Vandenput L, Van  
 575 Der Velde N, Viikari J, Xiao SM, Zhao JH, Evans DS, Cummings SR, Cauley J,  
 576 Duncan EL, De Groot LCPGM, Esko T, Gudnason V, Harris TB, Jackson RD,  
 577 Jukema JW, Ikram AMA, Karasik D, Kaptoge S, Kung AWC, Lehtimäki T,  
 578 Lyytikäinen LP, Lips P, Luben R, Metspalu A, Van Meurs JBJ, Minster RL,  
 579 Orwoll E, Oei E, Psaty BM, Raitakari OT, Ralston SW, Ridker PM, Robbins JA,  
 580 Smith A V., Stykarsdottir U, Tranah GJ, Thorstensdottir U, Uitterlinden AG,  
 581 Zmuda J, Zillikens MC, Ntzani EE, Evangelou E, Ioannidis JPA, Evans DM,

582 **Ohlsson C.** Large-scale GWAS identifies multiple loci for hand grip strength  
583 providing biological insights into muscular fitness. *Nat Commun* 8, 2017.

584 40. **Ye S.** An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic*  
585 *Acids Res* 29: 88e – 88, 2001.

586 41. **Yeung SSY, Reijnierse EM, Trappenburg MC, Hogrel JY, McPhee JS, Piasecki**  
587 **M, Sipilä S, Salpakoski A, Butler-Browne G, Pääsuke M, Gapeyeva H, Narici M**  
588 **V., Meskers CGM, Maier AB.** Handgrip Strength Cannot Be Assumed a Proxy for  
589 Overall Muscle Strength. *J Am Med Dir Assoc* 19: 703–709, 2018.

590 42. **Zillikens MC, Demissie S, Hsu YH, Yerges-Armstrong LM, Chou WC, Stolk L,**  
591 **Livshits G, Broer L, Johnson T, Koller DL, Kutalik Z, Luan J, Malkin I, Ried JS,**  
592 **Smith A V., Thorleifsson G, Vandenput L, Hua Zhao J, Zhang W, Aghdassi A,**  
593 **Åkesson K, Amin N, Baier LJ, Barroso I, Bennett DA, Bertram L, Biffar R,**  
594 **Bochud M, Boehnke M, Borecki IB, Buchman AS, Byberg L, Campbell H,**  
595 **Campos Obanda N, Cauley JA, Cawthon PM, Cederberg H, Chen Z, Cho NH,**  
596 **Jin Choi H, Claussnitzer M, Collins F, Cummings SR, De Jager PL, Demuth I,**  
597 **Dhonushe-Rutten RAM, Diatchenko L, Eiriksdottir G, Enneman AW, Erdos M,**  
598 **Eriksson JG, Eriksson J, Estrada K, Evans DS, Feitosa MF, Fu M, Garcia M,**  
599 **Gieger C, Girke T, Glazer NL, Grallert H, Grewal J, Han BG, Hanson RL,**  
600 **Hayward C, Hofman A, Hoffman EP, Homuth G, Hsueh WC, Hubal MJ,**  
601 **Hubbard A, Huffman KM, Husted LB, Illig T, Ingelsson E, Ittermann T, Jansson**  
602 **JO, Jordan JM, Jula A, Karlsson M, Khaw KT, Kilpeläinen TO, Klopp N, Kloth**  
603 **JSL, Koistinen HA, Kraus WE, Kritchevsky S, Kuulasmaa T, Kuusisto J, Laakso**  
604 **M, Lahti J, Lang T, Langdahl BL, Launer LJ, Lee JY, Lerch MM, Lewis JR,**  
605 **Lind L, Lindgren C, Liu Y, Liu T, Liu Y, Ljunggren Ö, Lorentzon M, Luben RN,**  
606 **Maixner W, McGuigan FE, Medina-Gomez C, Meitinger T, Melhus H, Mellström**

**D, Melov S, Michaëlsson K, Mitchell BD, Morris AP, Mosekilde L, Newman A,**  
**Nielson CM, O’Connell JR, Oostra BA, Orwoll ES, Palotie A, Parker SCJ,**  
**Peacock M, Perola M, Peters A, Polasek O, Prince RL, Rääkkönen K, Ralston SH,**  
**Ripatti S, Robbins JA, Rotter JI, Rudan I, Salomaa V, Satterfield S, Schadt EE,**  
**Schipf S, Scott L, Sehmi J, Shen J, Soo Shin C, Sigurdsson G, Smith S, Soranzo**  
**N, Stančáková A, Steinhagen-Thiessen E, Streeten EA, Styrkarsdottir U, Swart**  
**KMA, Tan ST, Tarnopolsky MA, Thompson P, Thomson CA, Thorsteinsdottir U,**  
**Tikkanen E, Tranah GJ, Tuomilehto J, van Schoor NM, Verma A, Vollenweider**  
**P, Völzke H, Wactawski-Wende J, Walker M, Weedon MN, Welch R, Wichmann**  
**HE, Widen E, Williams FMK, Wilson JF, Wright NC, Xie W, Yu L, Zhou Y,**  
**Chambers JC, Döring A, van Duijn CM, Econs MJ, Gudnason V, Kooner JS,**  
**Psaty BM, Spector TD, Stefansson K, Rivadeneira F, Uitterlinden AG, Wareham**  
**NJ, Ossowski V, Waterworth D, Loos RJJ, Karasik D, Harris TB, Ohlsson C,**  
**Kiel DP.** Erratum: Large meta-analysis of genome-wide association studies identifies  
 five loci for lean body mass. *Nat Commun* 8: 1414, 2017.

## Figure Legends

**Figure 1. Genotype versus Grip Strength for *TGFA* (rs958685; effect allele = A), *GLIS1* (rs4926611; effect allele = C) and *GBF1* (rs2273555; effect allele = A) in a mixed population of older elite athletes (sprint and endurance) and non-athletes.** Grip strength according to genotype for (A) *TGFA*, (B) *GLIS1* and (C) *GBF1* (irrespective of groupings). \*= $P < 0.05$  versus AA (multinomial logistic regression analysis).

**Figure 2. Genotype versus Total Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes.** Total Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings).

**Figure 3. Genotype versus Appendicular Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes.** Appendicular Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings).

**Figure 4. Genotype versus Percentage Lean Mass for *ADAMTSL3* (rs4842924; effect allele = T), *IRS1* (rs2943656; effect allele = A) and *FTO* (rs9936385; effect allele = T) in a mixed population of older elite athletes (sprint and endurance) and non-athletes.** Percentage Lean Mass according to genotype for *ADAMTSL3* (A), *IRS1* (B) and *FTO* (C; irrespective of groupings). \*\*= $P < 0.01$  versus CC (multinomial logistic regression analysis).

648

649 **Figure 5. Phenotype characteristics of older elite athlete (sprint and endurance) and**  
650 **non-athlete (Control) populations.** Total lean mass (A), appendicular lean mass (ALM; B),  
651 % lean mass (C), % fat mass (D), grip strength (E) and maximum power relative to body  
652 weight (F) in master athletes and non-athlete controls. \*\*\*= $P < 0.001$  (unpaired t test).

653

654 **Figure 6. Genotype distributions of selected single nucleotide polymorphisms (SNPs)**  
655 **previously associated with lean body mass or grip strength in master athlete (MA) and**  
656 **non-athlete (Ctrl) populations.** Balloon plot displaying frequencies of genotypes for three  
657 lean mass-associated SNPs (*IRS-1*, *FTO* and *ADAMTSL3*) and three grip strength-associated  
658 SNPs (*TGFA*, *GLIS1* and *GBFI*) between elite older athletes and non-athlete controls.  
659 \*= $P < 0.05$  (Fisher's exact test).

660

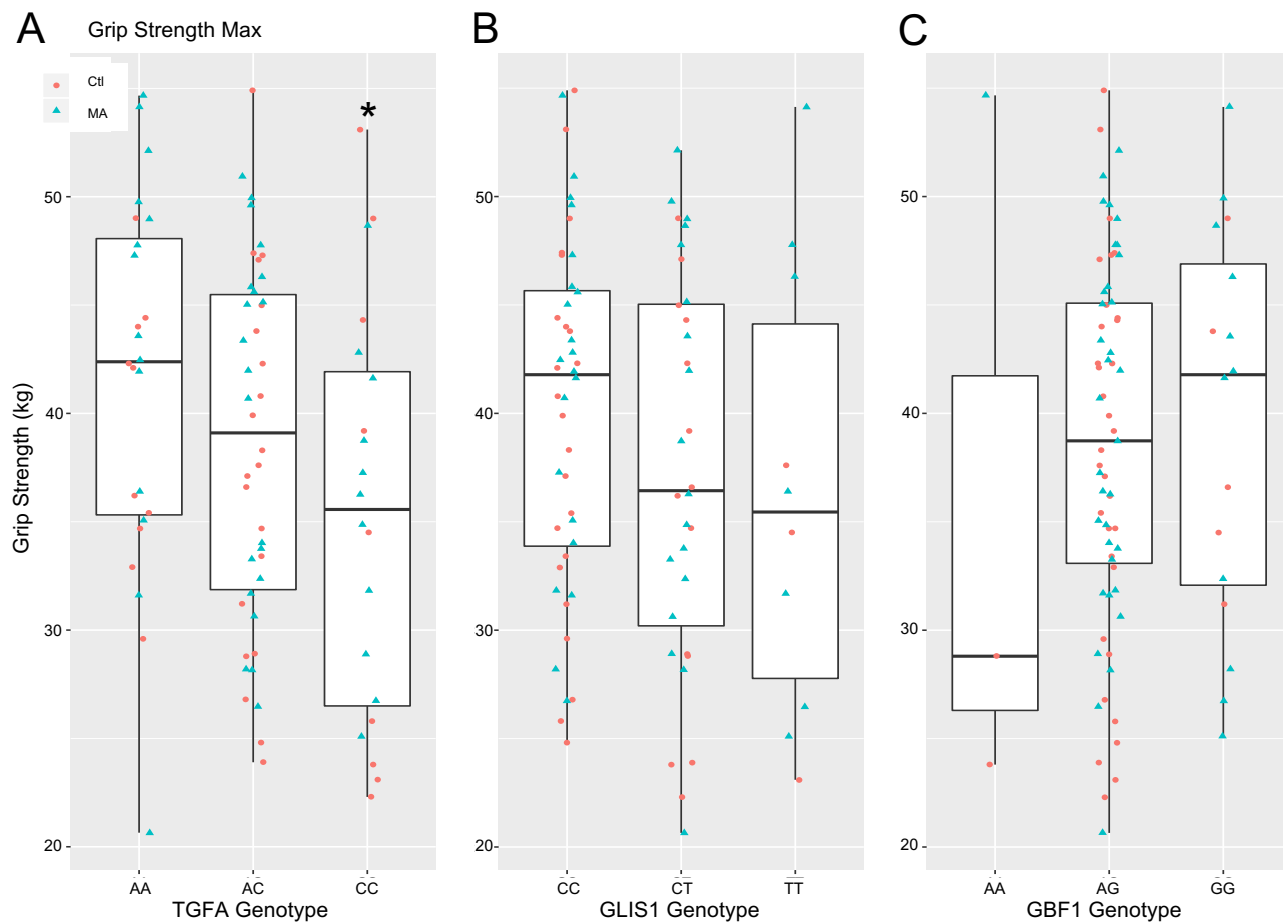


Figure 1

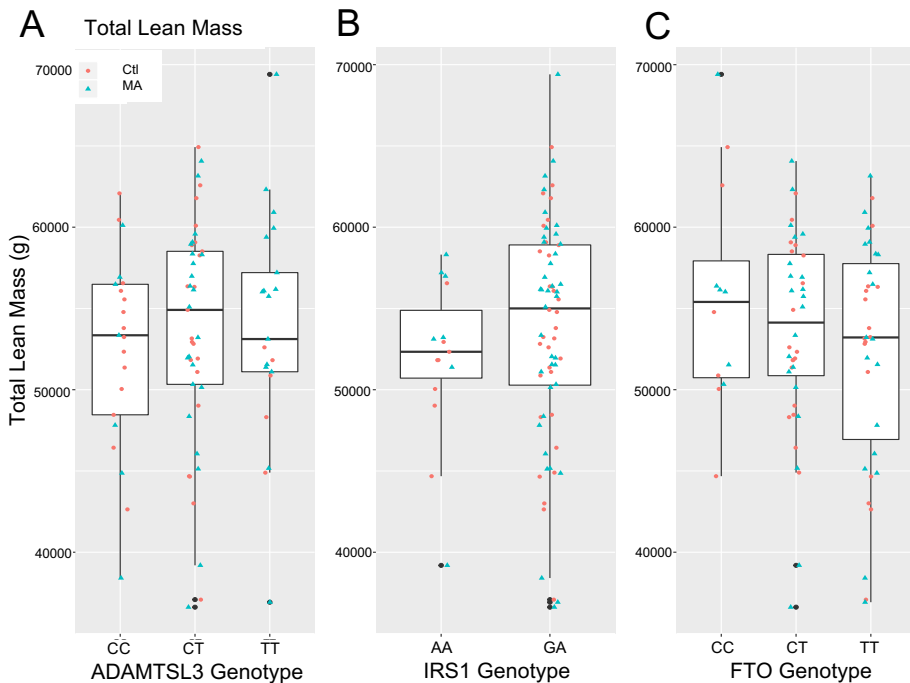
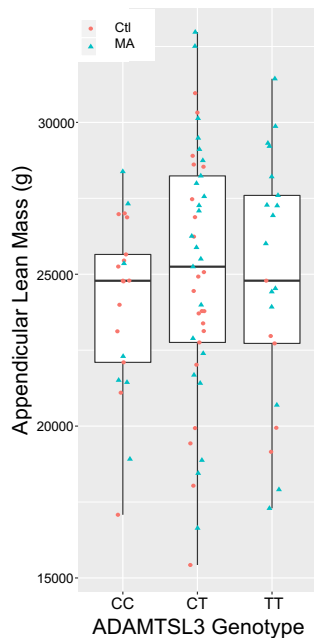
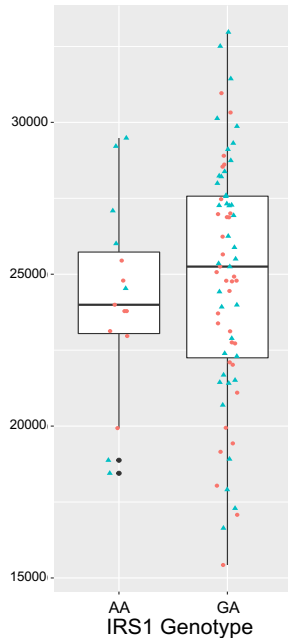
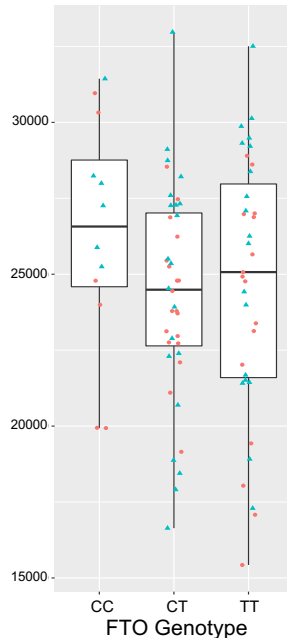
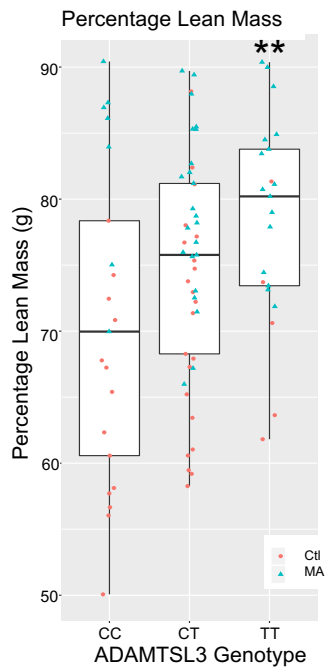
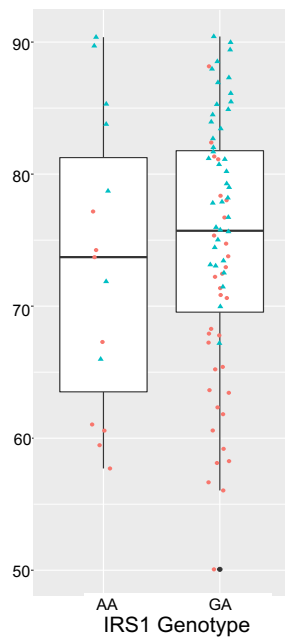
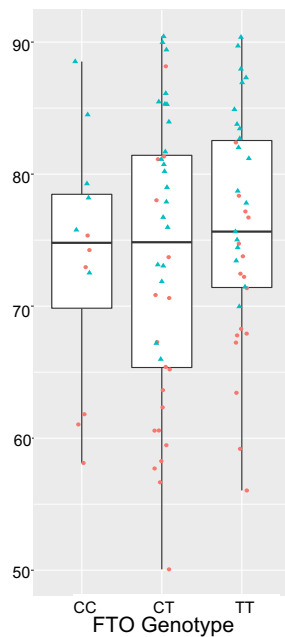


Figure 2



**A****Appendicular Lean Mass****B****C****Figure 3**

**A****B****C****Figure 4**

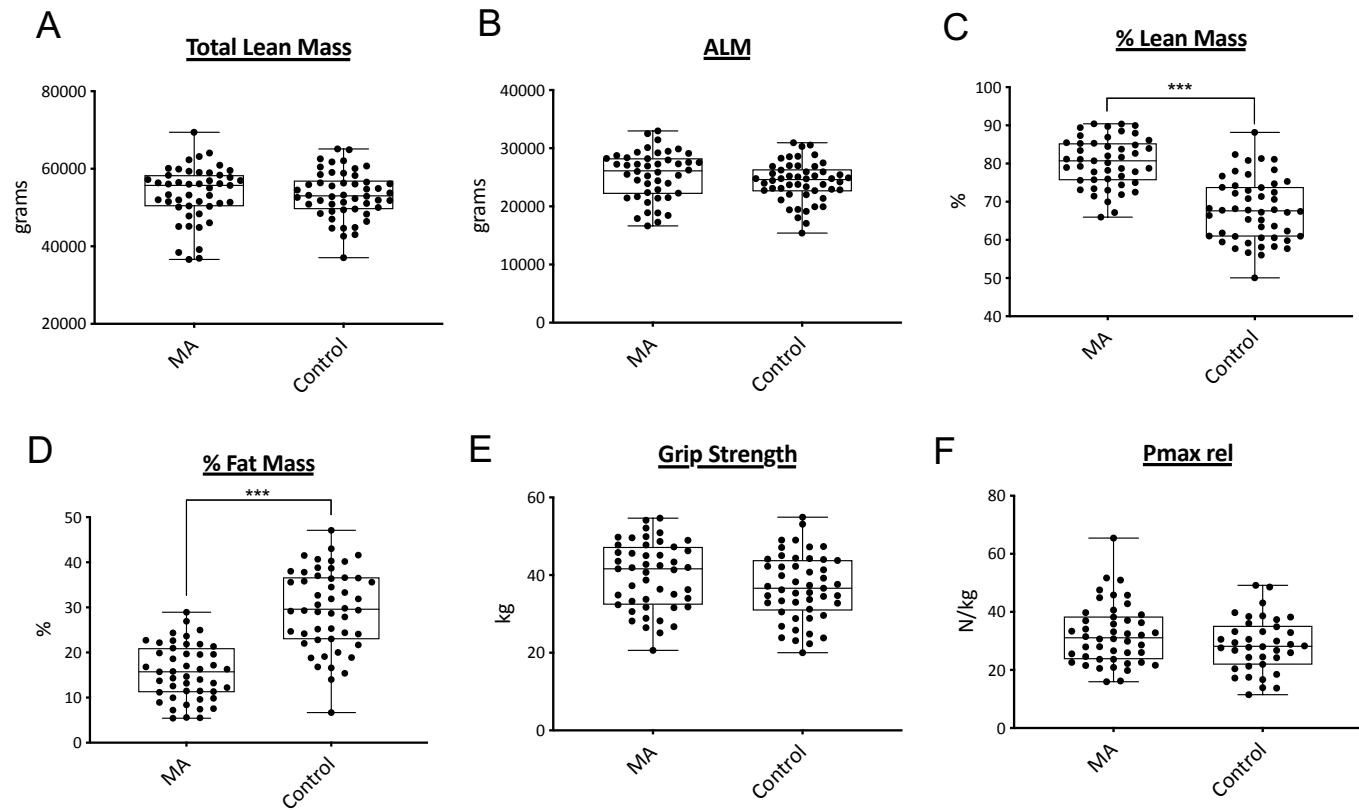


Figure 5

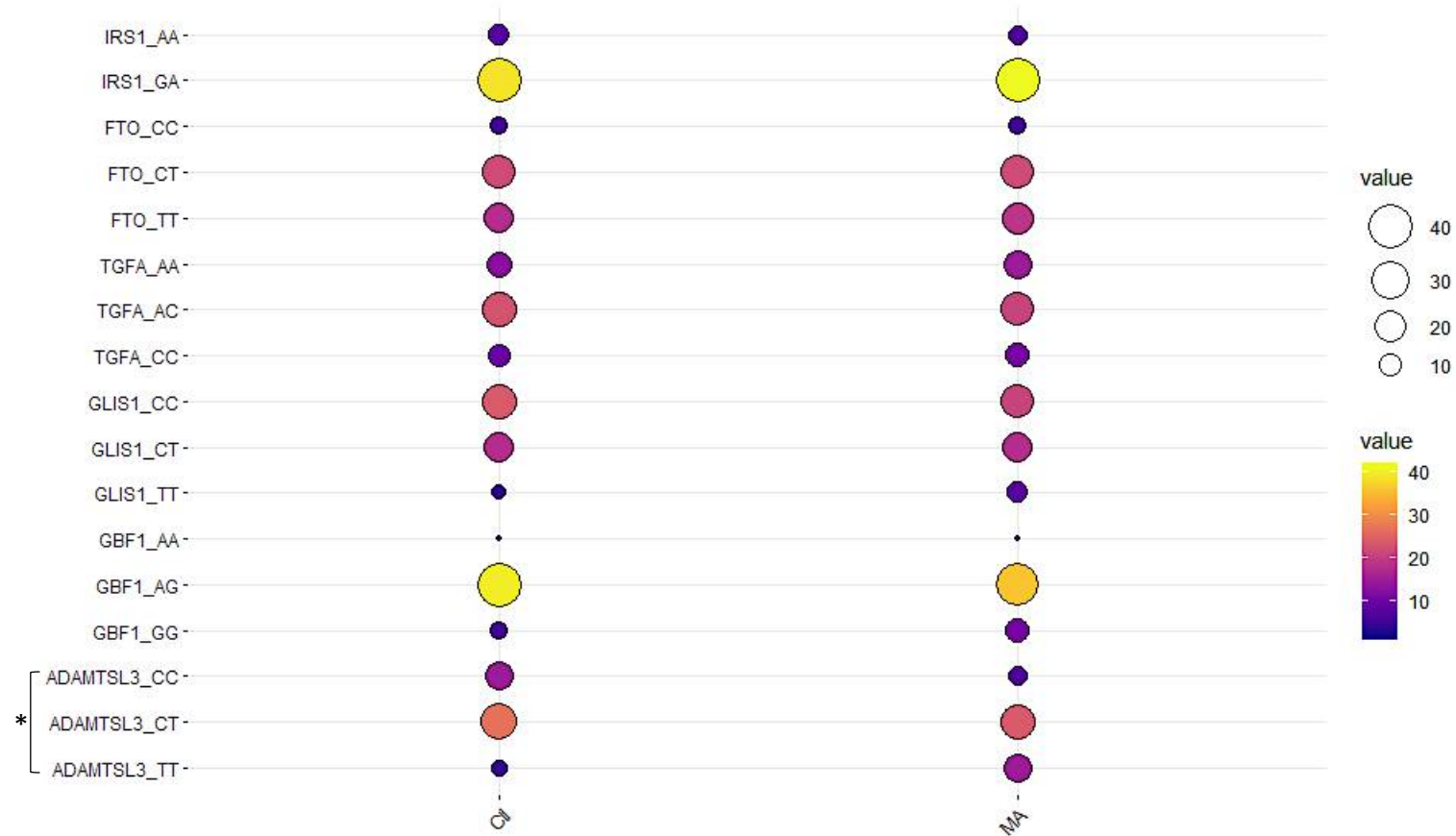


Figure 6

**Table 1: Single nucleotide polymorphism (SNP) and primer information for tetra-primer ARMS PCR.**

SNP	Closest Gene	Allele 1/2	EAF	Primer sequences (5'-3')	Annealing temperature	Ratio of FO:RO:RI:FI
rs2943656	IRS1	A/G	0.38	FO: CTGAGAGCCTGCTCCTTACTCTTGTCTT RO: CGGCATGTTGGAGAGTTACTCTACATGT FI: TTCACCTAAAATTCTCCTCTAAAAACACAG RI: CTCTCTCCATCACCATGGCTTCACCT	62°C	1:1:3:3
rs4842924	ADAMTSL3	T/C	0.52	FO: CAGTTGGAGTACTGAGAATGAGACAGGG RO: AGTCTTAGGACTCAGACTTGCCATCACA FI: GGAAAGGATAAGGATGTTGTGAGCGT RI: GAATAGGCAATAGCTTCTATGTGAGCG	61°C	2:1:6:2
rs9936385	FTO	T/C	0.61	FO: TGTGTGACCAGCCTCAATAGATTTTATTCA RO: CCATCCTATCAAAAACAGCACTCTCACC FI: TGCATATGAAGAGGGATTTTTTGCATC RI: TACTGGGAATATGCAGTGAACCACGA	62°C	1:1:3:3
rs958685	TGFA	A/C	0.52	FO: TCCACCCCTTAGGAAAAAATGCTTCCTCT RO: TCACATCTTTGTCATGGGACATAGTCCC FI: TTTTTCATCGGCAGTTTGCAGATACC RI: AGGAGTATCCTTCTCCACCCACGCT	62°C	1:2:2:6
rs2273555	GBF1	A/G	0.61	FO: CACAACCACAATGTTTCGTAACAGAAATG RO: TCTAAAACTGGGAAAGGAAGCAATGTG FI: TTTCTAAGTCCTATTTACTGAAAACCAAG RI: ACACTGAAGCCCCACCTAAGGAACGCT	61°C	1:1:3:3
rs4926611	GLIS1	C/T	0.64	FO: GCAGAGCTGGATTTTCAAGAGTCTACCT RO: TTCATCCCTGCTTACCCACTAGAGGTAA FI: TAGAGACACCTGCAACATCCAGCAAAT RI: CTGAGATTGCTTTTAAATTCAGCAGTG	61°C	1:2:3:6

**Table 2. Allele frequencies of selected single nucleotide polymorphisms (SNPs) previously associated with lean body mass or grip strength in individuals grouped according to the highest and lowest quartile for % LBM or HGS.**

SNP	Closest Gene	Allele 1/2	Lowest Quartile for %LBM		Highest Quartile for %LBM		SNP	Closest Gene	Allele 1/2	Lowest Quartile for HGS		Highest Quartile for HGS	
			Allele 1	Allele 2	Allele 1	Allele 2				Allele 1	Allele 2	Allele 1	Allele 2
rs2943656	<i>IRS1</i>	A/G	28	20	28	20	rs958685	<i>TGFA</i>	A/C	19	29	30	18*
rs4842924	<i>ADAMTSL3</i>	T/C	15	33	27	21*	rs2273555	<i>GBF1</i>	A/G	22	26	21	27
rs9936385	<i>FTO</i>	T/C	34	14	29	19	rs4926611	<i>GLIS1</i>	C/T	28	20	34	14

Frequencies of alleles for three lean mass-associated SNPs (IRS-1, FTO and ADAMTSL3) between the highest and lowest quartile for LBM (irrespective of groupings), and three grip strength-associated SNPs (TGFA, GLIS1 and GBF1) between the highest and lowest quartile for HGS (irrespective of groupings). \*=P<0.05 vs. Lowest Quartile (Fisher's exact test).

**Table 3. Allele frequencies of selected single nucleotide polymorphisms (SNPs) previously associated with lean body mass or grip strength in elite athletes (sprint and endurance) versus non-athlete controls.**

SNP	Closest Gene	Allele 1/2	Control (n=48)		Sprint (n=12)		Endurance (n=36)	
			Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
rs2943656	<i>IRS1</i>	A/G	57 (59%)	39 (41%)	13 (54%)	11 (46%)	42 (58%)	30 (42%)
rs4842924	<i>ADAMTSL3</i>	T/C	38 (40%)	58 (60%)	15 (62%)*	9 (38%)*	42 (58%)*	30 (42%)*
rs9936385	<i>FTO</i>	T/C	60 (62%)	36 (38%)	10 (42%)	14 (58%)	51 (71%)	21 (29%)
rs958685	<i>TGFA</i>	A/C	51 (53%)	45 (47%)	13 (54%)	11 (46%)	39 (54%)	33 (46%)
rs2273555	<i>GBF1</i>	A/G	44 (46%)	52 (54%)	7 (29%)	17 (71%)	31 (43%)	41 (57%)
rs4926611	<i>GLIS1</i>	C/T	68 (71%)	28 (29%)	15 (62%)	9 (38%)	46 (64%)	26 (36%)

Frequencies of alleles for three lean mass-associated SNPs (IRS-1, FTO and ADAMTSL3) and three grip strength-associated SNPs (TGFA, GLIS1 and GBF1) between non-athlete controls and elite athletes (split into sprint and endurance types). \*= $P < 0.05$  vs. Control (Fisher's exact test).